

Signaling

Major finding: Phosphorylation of wild-type BRAF on Ser729 by AMPK suppresses MEK/ERK signaling.

Mechanism: BRAF Ser729 phosphorylation disrupts binding to KSR1 and promotes binding to 14-3-3.

Impact: AMPK activators may prevent paradoxical ERK activation by BRAF inhibitors in *BRAF*-wild-type cells.

AMPK PHOSPHORYLATES AND NEGATIVELY REGULATES BRAF

AMP-activated kinase (AMPK) is a critical regulator of energy homeostasis that is activated in response to low cellular energy levels. AMPK is thought to coordinate cellular energy status with cell growth and proliferation, but the underlying mechanisms are not completely clear. Shen and colleagues found that AMPK significantly attenuated MEK-ERK signaling in *BRAF*-wild-type cells by physically associating with BRAF and directly phosphorylating serine 729 (Ser729), which in turn led to decreased ERK activation. Phosphorylation of Ser729 had no effect on BRAF kinase catalytic activity, but blocked BRAF-mediated signaling by promoting an inhibitory interaction between BRAF and 14-3-3 proteins and disrupting the association between BRAF and the scaffolding protein KSR1, which facilitates phosphorylation of MEK by RAF. AMPK activation blocked cell cycle progression in keratinocytes expressing wild-type BRAF, whereas mutation of the Ser729 residue led to a significant increase in proliferation rates, further indicating that phosphorylation of Ser729 by AMPK negatively regulates BRAF-dependent mitogenic signaling. Given

these findings, the authors hypothesized that AMPK activation might be a potential strategy to combat paradoxical MEK-ERK activation caused by BRAF inhibitors in *BRAF*-wild-type cells, a phenomenon that leads to the development of secondary cutaneous squamous cell carcinomas and keratoacanthomas in some patients with *BRAF*-mutant metastatic melanoma. Indeed, combined treatment with the AMPK activator phenformin and the BRAF inhibitor PLX4720 significantly reduced epidermal hyperplasia in mice in association with reduced ERK phosphorylation. The identification of AMPK as a BRAF Ser729 kinase provides a link between cellular energy status and proliferation and raises the possibility that combined use of AMPK activators with BRAF inhibitors may mitigate the paradoxical MEK-ERK activation that has been associated with secondary malignancies and drug resistance. ■

Shen CH, Yuan P, Perez-Lorenzo R, Zhang Y, Lee SX, Ou Y, et al. Phosphorylation of BRAF by AMPK impairs BRAF-KSR1 association and cell proliferation. *Mol Cell* 2013 Oct 3 [Epub ahead of print].

Epigenetics

Major finding: *NSD1* mutations are recurrent in pediatric ALL and induce a distinct chromatin signature.

Approach: Quantitative mass spectrometry was used to measure bulk histone modification levels across cell lines.

Impact: Chromatin profiling can reveal the epigenetic consequences of genetic or chemical perturbations.

CHROMATIN PROFILING UNCOVERS A RECURRING MUTATION IN PEDIATRIC ALL

The epigenomes of cancer cells are commonly altered, but efforts to comprehensively study epigenetic changes in cancer cells have been hindered by the limited availability of antibodies capable of recognizing specific histone modifications. Jaffe and colleagues devised a mass spectrometry-based method to simultaneously quantify levels of multiple histone modifications in bulk chromatin and used this technique to profile 42 distinct histone H3 modifications in a subset of hematologic cancer cell lines in the Cancer Cell Line Encyclopedia (CCLE). Unsupervised clustering grouped the data into six chromatin states, with one distinguished by increased H3 lysine 36 dimethylation (H3K36me2) and lower levels of unmodified H3K36. Six of the thirteen cell lines in this cluster harbored a t(4;14) translocation that leads to the overexpression of the H3K36 methyltransferase nuclear receptor SET domain-containing 2 (*NSD2*; also known as MMSET). However, the remaining cell lines did not have a t(4;14) translocation, prompting an analysis of the genomic features of these cell lines. Remarkably, each one harbored an *NSD2* coding variant that resulted in an E1099K substitution



in the *NSD2* SET domain. The E1099K protein had increased methyltransferase activity *in vitro*, and its expression in an *NSD2*-deficient cell line increased H3K36me3 dimethylation in association with increased anchorage-independent growth. *NSD2* mutations were enriched in pediatric acute lymphoblastic leukemia (ALL) cell lines in the CCLE, and growth and tumorigenicity of these cells were dependent on *NSD2*. Exon sequencing of *NSD2* in a larger set of pediatric cancers identified *NSD2* mutations in 7.5% of B-cell ALLs (B-ALL) overall and 20% of *ETV6-RUNX1*-positive B-ALL samples. These findings, which implicate *NSD2*-dependent H3K36 hypermethylation as a driving event in a subset of pediatric ALLs, highlight the potential of mass spectrometry chromatin profiling to rapidly uncover epigenetic consequences of cancer-associated mutations. ■

Jaffe JD, Wang Y, Chan HM, Zhang J, Huether R, Kryukov GV, et al. Global chromatin profiling reveals *NSD2* mutations in pediatric acute lymphoblastic leukemia. *Nat Genet* 2013 Sept 29 [Epub ahead of print].